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CARBON REQUIREMENTS OF *GLŒOSPORIUM PSIDII* CAUSING DIE-BACK OF GUAVAS

BY R. N. TANDON AND R. K. AGARWALA

(*Department of Botany, University of Allahabad*)

Read on December 28, 1954

INTRODUCTION

IT has long been established that carbon as well as a number of other elements are indispensable for the growth of fungi. According to Weimer (1924), Harter (1921), Young and Barnett (1922), Leonian (1924), Leclerg (1930) saccharose, glucose and fructose were excellent sources of carbon for many fungi. Saksena and Mehrotra (1949) noted that dextrose, maltose, sucrose and soluble starch were most favourable for the growth of the genus Pythium. Arabinose, rhamnose, xylose, galactose, mannose, lactose, dextrins, glycogen, inulin and glycerine were utilized only by some of them. Different organisms show a different utilization of various carbon compounds. Hawker (1939) has shown the effect of various carbon compounds on the formation of perithecia by *Melanospora destruens* in the presence of accessory growth factors. Lilly and Barnett (1951) considered the effect of mixed carbon sources as highly specific on the growth of many fungi.

In the present instance the authors have tried to investigate quantitatively the carbon requirements of *Glœosporium psidii* and also the effect of mixed carbon compounds of glucose and starch in different quantities with a view that it would be helpful in dealing with various aspects of the guava anthracnose problem.

MATERIAL AND METHOD

The material for isolation and the method employed for it were the same as described by the authors in their previous paper (1954). The organism was grown in 150 ml. pyrex flask and the basal medium which will be referred to as Asthana and Hawker's medium A consists of the following substances: Glucose 5 gm. KNO_3 3·5 gm., KH_2PO_4 1·75 gm., $MgSO_4$ ·75 gm., Distilled water 1000 c.c. The amount of carbon in various media was similar to that present in 5 gm. of glucose. Liquid media were used throughout. These were prepared with reagent grade chemicals and distilled water. Cultures were grown at a constant temperature of 26° C. unless otherwise stated and the pH of various solutions was adjusted to vary between 4·8 and 5. Generally three replicates were used in each case. Filtration and drying process after 3 weeks' incubation was the same as described by Saksena and Mehrotra (1949).

EXPERIMENTAL RESULTS

Seventeen saccharides, glucosides and alcohols were tested at a carbon level which was equivalent to 2000 mgm./lit. Results are summarized in Table I.

Growth on mannitol and dulcitol was excellent, that on tartaric acid, starch, raffinose, galactose, sorbitol, sucrose, rhamnose, glucose and inulin was good. It was fair on lactose, dextrine and malic acid and poor on glycerine. No growth occurred on the basal medium which was lacking carbon.

The effect of carbon compounds in the basal medium on sporulation, appressoria and setæ formation was quite interesting. Glucose, galactose and tartaric acid recorded the best sporulation while mannitol, lactose, dextrin, maltose and starch showed fair sporulation and the rest gave very poor sporulation. Tartaric acid, maltose, dextrine, dulcitol, did not show appressorial formation. Setæ formation was not a regular feature and it varied on various compounds. The colour of appressoria and the setæ was also changed with the change of the compound.

In nature the fungi usually come in contact with mixed carbon sources rather than a single source of carbon. Certain fungi make more growth when supplied with a mixture of carbon compounds. Horr (1936) investigated the growth of *A. niger* on a mixture of glucose and galactose. Brown (1925) used starch and glucose as the carbon source for the growth of Fusarium. Lilly and Barnett (1951) considered the effect of mixed carbon sources as highly specific on the growth of many fungi. An attempt has,

TABLE I

Showing Dry Weight Yields, Sporulation and the Spore Size on Various Carbon sources Added singly at the rate of 2000 mgm. Carbon per litre

Compound	Source	Yield	Sporulation	Appressoria	Setæ and its colour
1. Rhamnose	Kerfoot & Co., Lancashire	40	Rare	Auburn	No
2. Sorbitol	B.D.H.	58	"	Dark Brown	No
3. Mannitol	"	103	"	Milkado Brown	No
4. Sucrose	"	43·6	* *	Auburn	Very long, Olivaceous Black
5. Galactose	E. Merck	55·5	* * *	Vendyke Brown	No
6. Lactose	B.D.H.	36·3	*	Auburn	Olivaceous black
7. Dulcitol	"	84·7	Rare	No	Irony Grey
8. Raffinose	Kerfoot	54	*	Milkado Brown	No
9. Glucose	C.P.C., U.S.A.	44	* * * *	Auburn	Olivaceous black
10. Dextrin	E. Merck	34	*	No	"
11. Glycerine	"	19	Rare	Auburn	No
12. Maltose	"	45·6	* * *	No	Steel Grey
13. Soluble starch	"	54	* *	Few Auburn	No
14. Inulin	Colman & Bell	50	Rare	Blister Colour	No
15. Tartaric Acid	Int. Ch. W.	64	"	No	Irony Grey
16. Malic Acid	—	28	"	Milkado Brown	No
17. No Carbon	—	No growth	—	—	—

* Poor ** Fair *** V. fair **** Good

therefore, also been made to find out whether the mixed carbon compounds have any effect on the growth and other characters of the organism.

Brown's starch medium has been varied in the same ratio which has been used by Brown in (1925) to find out the effect of simultaneous variation of Glucose and Starch. The results are recorded in Table II.

TABLE II

Showing the Dry Weight, Yeild, Sporulation and the Effect on the Formation of Appressoria and Setæ, etc.

Glucose in %	Starch in %	Sporulation	Yield	Appressoria	Setæ and its colour
0·2	0·0	Rare	29·0	No	No
0·4	0·0	*	45	Auburn	Irony Grey
0·2	0·2	**	58	Blister Colour	No Setæ
0·7	0·0	**	78	,,	,,
0·2	0·5	***	88	Milkado Brown	,,
1·2	0·0	Rare	170	Vendyke Brown	Olivaceous Black
0·2	1·0	****	98·5	Few Auburn	No

* Poor ** Fair *** V. fair **** Good

It has been observed that different carbon concentrations have marked effect on the growth of the fungus. The increasing concentrations of glucose alone without any starch gave increased mycelial growth but after a minimum quantity of glucose the sporulation decreased with the increased concentration of glucose. At 1·2% glucose concentration the growth was very luxuriant but sporulation was as rare as on 0·2% glucose.

When starch was added, the vegetative growth was only slightly better than when the same amount of glucose was replaced with similar quantities of starch and glucose. The increase in the mycelium was not much but the sporulation was distinctly better. Glucose alone gave profuse growth of mycelium but sporulation was poor. The mycelium was much distorted on higher concentrations of glucose. Olive buff-coloured chlamydospores were observed throughout the various series of glucose and starch.

Discussion

The physico-chemical conditions or nutrient components seem to influence the growth of the fungus very effectively. The results obtained with carbon sources and the mixed carbon compounds afford some basis for the development of differential media. Mannitol and dulcitol supported best growth. However, the sources were far superior to glucose which is probably the best carbon source for most fungi. Glucose gave abundant growth only when its concentration was increased. Rhamnose has generally been reported as a poor source of carbon by Farries and Bell (1930). The authors have noted the fact that *Glœosporium psidii* could utilize this compound fairly well. It was also observed that hexoses gave fair growth though Bhargava (1945) found galactose to be useless as a source of carbon for *Achlya* Sp.

Amongst the disaccharides maltose and sucrose served as a good source of carbon for Glœosporium. Lactose did not favour much growth of the fungus. Raffinose (Trisaccharide), starch and inulin gave very good growth while tartaric acid was favoured most by the fungus under investigation. Malic acid, dextrine and glycerine did not support good growth of the fungus.

It was further noted that carbon is essential for this fungus. No growth could be observed in its absence and the mycelial growth was noticed to increase upto a certain extent with the increased doses of glucose and starch separately or together (*vide* Table II). After the optimum concentration it again continued to decrease. The sporulation was better when starch was mixed with glucose. The general effect of increasing the carbon was to increase the density of growth of the aerial mycelium. Thus the effect of increasing the carbon of a medium was more or less similar to that produced by simple concentrations of that medium.

Summary

Glœosporium psidii grew best on mannitol and dulcitol. Its growth on tartaric acid, starch, raffinose, galactose, sorbitol, sucrose, rhamnose, glucose and inulin was fairly good; while it was comparatively poorer on malic acid, dextrine and glycerine.

The vegetative growth was luxurious at 1·2% glucose. Best sporulation was obtained with starch. Maltose and sucrose did not favour sporulation though they produced good vegetative growth. Appressoria and setæ did not remain consistent in their colour on various carbon compounds.

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AMBASSIS RANGA, H.B., A NEW HOST OF *ARGULUS* SP.

By R. B. Malaviya

(*Department of Zoology, Mahakoshal Mahavidyalaya, Jabalpur, M.P.*)

Read at the Annual Meeting on 28th December 1954

(Communicated by Dr. H. R. Mehra)

Introduction

A detailed survey of the fish fauna of Robertson Lake situated in the vicinity of Mahakoshal Mahavidyalaya, four miles east of Jabalpur (M.P.), has been undertaken since July 1954, with a view to study the distribution and ecology of certain species of fresh-water fishes. During this survey an interesting case of infection of *Ambassis ranga*, H.B. with the fish-louse (*Argulus*) was observed and is reported here for the first time.

Argulus foliaceous Linnæus has been known to parasitise several species of carps in India. The parasite was described by Southwell (1915) for the first time from the skin of *Labeo rohita*. Hora (1943) observed a heavy infestation of *Labeo rohita, Catla catla* and *Cirrhina mrigala* by this parasite in the Churchuria Fishery, Dhapa. Khan in a paper in 1944 reported the parasite from the skin of *Cirrhina mrigala* from a tank at Lyallpur. In the same paper he referred that Hora in 1933 recorded this parasite from the skin of *Ophiocephalus* living in a breeding tank in Dharangadhara State.

Observations

On 20th September 1954, three specimens of *Ambassis ranga* were collected along with many other fishes. One of the *Ambassis ranga* was found to be infected with *Argulus* just at the base of the caudal fin on the left side of the fish. All the three specimens of *Ambassis ranga* were kept in the Departmental aquarium for further observations. It was observed that the infected fish never manifested the characteristics of a normal living fish. It was erratic in movements at intervals, generally floated to the top, and turned over at times. The parasite was removed carefully from the place of infection and on removal of the parasite the skin of the fish was found to be worn out at the place of attachment and fin-rays were seen exposed. There was a heavy damage caused to the tissues in the region of infection.

Conclusion

Ambassis ranga is an Acanthopterygian fish belonging to the family Percidæ (Day, 1889) which are almost marine fishes, the exception being

few species of *Ambassis* which are fresh-water inhabitants. *Ambassis ranga* is found throughout India and Burma. In Madhya Pradesh it is thoroughly distributed in many rivers and ponds as reported by D' Abrue (1936). It is abundant in Robertson Lake, Jabalpur.

Argulus foliaceous has been reported to cause heavy mortality among carp fisheries in Bengal and elsewhere in India and is primarily ectoparasitic on them. *Ambassis ranga* is a small fish and quite bony, hardly attaining 3–4″ in length and is also not good eating. Moreover, it is also not suitable for fish culture. Though its infection by *Argulus* is not directly of much harm as far as the food value of this fish is concerned, yet the infection of *Ambassis ranga* with *Argulus* is to be feared in those ponds which abound in carps and other fishes of nutritional value. Moreover, its presence is equally dangerous in fish farms and stocking ponds which form the potential reserves of our fisheries.

Further investigations regarding the infection of other fishes of food value with this parasite are in progress.

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EFFECT OF SOME NITROGEN COMPOUNDS ON THE GROWTH AND SPORULATION OF *PHYLLOSTICTA CYCADINA* (PASSER)

BY R. N. TANDON AND K. S. BILGRAMI

(*Department of Botany, University of Allahabad*)

Received on December 1, 1954

NITROGEN has a marked effect on the nutrition of various fungi. Robbins (1937) pointed out that on the basis of their nitrogen requirements the fungi could be classified into four groups. According to him the first group utilized organic nitrogen alone, the second group both organic nitrogen and ammonia, the third not only organic nitrogen and ammonia but also nitrate nitrogen; while the fourth group was capable of fixing even elemental nitrogen. Mix (1933) found that potassium nitrate was the best nitrogenous compound for the growth of *Phyllosticta solitaria.* Tandon (1950) observed that the growth of *Pestalotia psidii* was good when acetamide or peptone was used as a source of nitrogen. Brock (1951) has reported that for *Morchella esculenta* nitrite was better source of nitrogen than nitrate. Tandon and Agarwal (1954) observed that sodium nitrite was more suitable for the growth of *Fusarium cœruleum* than ammonium salts. According to Wenck, Peterson and Fred (1935) *Aspergillus fischeri* could utilize ammonium or organic nitrogen. Leonian and Lilly (1938) mention that *Saprolegnia parasitica* needed amino acids for their nitrogen requirement.

A review of the existing literature fully confirms that the fungi belonging to all the different groups showed well marked specificity for the kind of nitrogen compound they could utilize. It was, therefore, thought desirable to study the nitrogen requirements of *Phyllosticta cycadina.*

MATERIAL AND METHOD

The fungus was isolated from the infected leaflets of *Cycas revoluta* growing in the Botany Department of Allahabad University.

Throughout this investigation Pyrex glass wares and purest available chemicals were used. Fifty c.c. of nutrient solution was placed in 150 c.c. conical flasks and was sterilized in an autoclave at 15 lb. pressure for 15 minutes. For each series four replicates were used and inoculations were made by agar disc method. The fungus was allowed to grow for three weeks. At the end of this period the fungal mats of each flask were collected separately on previously dried and weighed Whatman's filter-paper No. 42. The fungus was dried in electric oven at 65° C. and was transferred to a desiccator

before weighing it quickly. This constant weight which was mostly obtained after three days was used as a quantitative measure for the growth of the fungus on the different nitrogen compounds. Asthana and Hawker's medium A* was selected as the basal medium. The amount of nitrogen in KNO_3 of Asthana and Hawker's medium A was calculated and it was replaced by equal quantity of nitrogen in the following organic and inorganic compounds.

Organic Compounds.—Acetamide, urea, peptone and asparagin.

Inorganic Compounds.—Sodium nitrate, calcium nitrate, magnesium nitrate, potassium nitrate, sodium nitrite, ammonium chloride, ammonium carbonate, ammonium nitrate and ammonium sulphate.

Previous investigation had established that the growth of *P. cycadina* was best at pH 5·2 and thus the pH of different modified media was adjusted to the same value.

Nitrogen compounds present in the leaves of *Cycas revoluta* were also estimated and the following results were obtained.

1.	Amides ..	.. 0·336 gm. per 100 gm of leaves			
2.	Nitrates and nitrites (Mixed) ..	.. 0·0252	,,	,,	,,
3.	Ammonia ..	.. Zero	,,	,,	,,
4.	Other free bases	.. Zero	,,	,,	,,

OBSERVATIONS

Both macroscopic and microscopic characters were recorded.

Macroscopic Characters.—It was observed that the growth was uniform on sodium nitrate, potassium nitrate, calcium nitrate, asparagin and peptone; but the thickness of the mat varied considerably. The growth was patchy on magnesium nitrate and urea. There was no growth when nitrogen was completely absent or when sodium nitrite was supplied to the medium. The growth was uniform on all the ammonium compounds, but the fungal mat was very thin.

Microscopic Characters.—Sodium nitrate—Mycelium was thin, septate with granular protoplasm. Abundant chlamydospores were present in chains. The colour of the mycelium, and the chlamydospores was light brown. The spores were small, oval, hyaline, and of uniform size (*vide* Fig. 1). Pycnidia were of dark brown colour with reticulate thickening on their walls. Their size varied considerably. It was also observed

* Glucose 5 gm., KNO_3 3·5 gm., KH_2PO_4 1·75 gm., $MgSO_4$ 0·75 gm., Distilled water 1 litre.

that the size of ostioles was proportional to the size of the pycnidia. Similar microscopic characters were obtained on the nitrates of calcium, potassium and magnesium. The hyphæ were thicker on acetamide and urea but other microscopic structures were similar to those on sodium nitrate. The mycelium on all the ammonium compounds was very thin and the hyphæ were greatly intertwined with each other (*vide* Fig. 2). Chlamydospore formation was rare on peptone while on asparagin they were formed in abundance. No chlamydospores were observed on the media containing ammonium nitrate and ammonium carbonate and only few were produced on ammonium sulphate or ammonium chloride.

The dry weight and other microscopic characters are recorded in Table I.

TABLE I

Showing dry weight and other microscopic characters of P. cycadina *on different nitrogen compounds*

Nitrogen compounds	Dry weight in gm.	Sporulation	Thickness of mycelium in μ	Size of chlamydospores in μ
Sodium nitrate ..	·0862	Good	6·6	6·6×6·3
Calcium nitrate ..	·1210	Poor	5·3	8·4× 7·3
Magnesium nitrate ..	·0782	Good	3·3	7·6× 5·9
Potassium nitrate ..	·0892	Good	4·6	12·9×11·3
Sodium nitrite ..	Nil	Nil	..	..
Acetamide ..	·1192	Poor	7·9	9·9× 8·6
Urea	·085	Good	8·3	12·9×10·6
Peptone	·0938	Fair	3·6	6·6× 5·6
Asparagin	·095	Fair	6·9	9·9× 8·6
Ammonium chloride..	·0622	Nil	2·9	3·9× 2·9
Ammonium nitrate ..	·0590	,,	4·3	..
Ammonium sulphate	·0644	,,	3·9	3·9 × 3·11
Ammonium carbonate	·0438	,,	3·6	..

The table indicates that the best vegetative growth of the organism was obtained on acetamide and calcium nitrate but sporulation was poor. These were followed by asparagin and peptone but even on them the sporulation was not good. The third series included sodium nitrate, magnesium nitrate,

potassium nitrate and urea where the growth was not so good but the sporulation was good. The growth was very poor on all the ammonium compounds. It is also clear from the table that the difference in the maximum and minimum thickness of the hyphæ was about four times. The average thickness of mycelium on urea was $8 \cdot 3\,\mu$ while on ammonium chloride it was only $2 \cdot 9\,\mu$. Largest chlamydospores were developed on urea and KNO_3, *i.e.*, $12 \cdot 9 \times 10 \cdot 6\,\mu$ and $12 \cdot 9 \times 11 \cdot 3\,\mu$ respectively and smallest on ammonium chloride, *i.e.*, $3 \cdot 9 \times 2 \cdot 9\,\mu$. These investigations also indicate that the growth on calcium nitrate was greater than on other nitrates. It was also found that addition of calcium chloride to the basal medium increased growth upto a concentration of $0 \cdot 125\%$ after which the growth decreased. Similar results were obtained with calcium phosphate where the growth increased upto $0 \cdot 25\%$ before showing any adverse effect of this substance. It appears that upto a limit calcium is capable of improving the growth and the best results obtained with calcium nitrate may be connected with the joint effect of calcium and nitrate.

As the ammonium compounds did not support good growth it was decided to study the effect of ammonia vapours on the germination of the spores of *Phyllosticta cycadina.* It was found that they failed to grow after an exposure to ammonia vapour for 1 hour and 25 minutes.

The effect of different concentrations of KNO_3 was also studied and it was found that there was no marked difference in the macroscopic and microscopic characters at different concentrations. Both the macroscopic and microscopic characters were similar to those described for sodium nitrate. It may be mentioned that the thickness of the mycelium and the size of the chlamydospores or pycnidia were not dependent on the amount of KNO_3 as they varied widely on the same concentration as well as on different concentrations.

The dry weight was found to increase with the increase of KNO_3 upto $0 \cdot 7\%$ but any further increase in its quantity caused a sudden decrease in its dry weight.

DISCUSSION

The importance of nitrogen in the growth of *Phyllosticta cycadina* was exhibited by the fact that there was no growth if nitrogen was not supplied to nutrient media. This showed that *P. cycadina* was incapable of fixing atmospheric nitrogen. Taking everything into consideration, it belongs to third group of the classification of Robbins (1937). Many workers including Young and Bennett (1922), Neal, Wester and Gunn (1933) reported

nitrates to be good source of nitrogen for the fungi used by them. In the present investigations also, nitrates were found to be satisfactory source of nitrogen.

Nitrite nitrogen was toxic for the growth of this organism and in this respect it was similar to numerous other fungi investigated by various persons. Talley and Blank (1942) working with *Phymatotrichum omnivorum*, Tandon and Agarwal (1954) for *Fusarium cœruleum* and Brock (1952) for *Morchella esculenta* have, however, reported nitrite to be satisfactory source of nitrogen for those fungi.

Ammonium salts were not satisfactory as they supported poor growth and vapours of ammonia were lethal for this fungus.

All the organic nitrogen compounds used in the present investigation supported very good growth which was best on acetamide. The host analysis also indicated that amides were present in large quantities and this may be the cause of good growth of this organism on leaflets of *Cycas revoluta*.

Brown (1925) working with a number of *Fusarium* strains observed that colour changes were brought about by change in the nutrients. A comparative study of the microscopic characters clearly indicated that changes in the source of nitrogen did not effect the size, shape and the colour of the spores which were fairly constant.

Summary

Phyllosticta cycadina was grown on a number of nitrogen compounds and it was established that the source of nitrogen greatly influenced the amount of growth. Nitrates were generally satisfactory and calcium nitrate supported best growth. There was no growth in complete absence of nitrogen or when it was supplied as a nitrite. Concentrations of KNO_3 beyond 0·7% reduced growth. Few organic nitrogen compounds were also used and out of them acetamide was found to be most suitable.

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* Original not consulted.

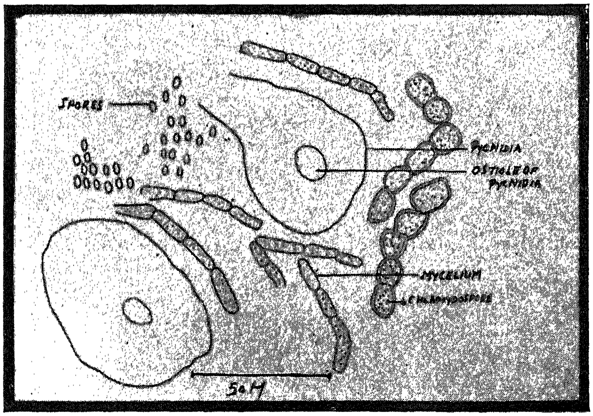
SPORES
PYCNIDIA
OSTIOLE OF PYCNIDIA
MYCELIUM
CHLAMYDOSPORE
50 µ

FIG. 1

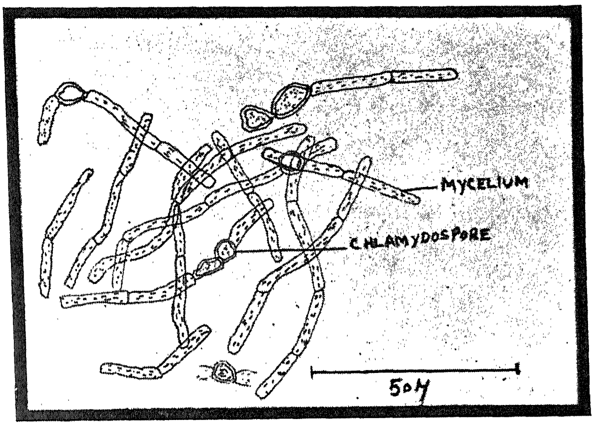
MYCELIUM
CHLAMYDOSPORE
50 µ

FIG. 2

GERMINATION OF SEEDS OF THREE COMMON WEEDS OF DRY PHASE OF LOW-LYING LANDS

BY L. P. MALL

(*Department of Botany, University of Saugar, Saugar*)

[Communicated by R. Misra, Ph.D. (Leeds), F.N.A.Sc.]

A DISTINCT Angiospermic flora appears in small pools and puddles in Oct-Nov. after a complete drying of rain-water in them. The seeds of such plants maturing in the late dry phase get submerged and evidently remain embedded in the mud during the monsoon period. Hence it is interesting to reveal the extent to which germination of preconditioned seeds is responsible for the growth of a characteristic vegetation of the drying beds of pools. Species like *Heliotropium supinum* Linn., *Mollugo hirta* Thumb. and *Polygonum plebejum* var. *Griffithii* are most common having a frequency of more than 80% in the community. Therefore these three species were selected for germination studies.

Mature and fully dry seeds of the above named species were collected in April, 1953 and April, 1954. The seeds of the latter year were subjected to the following pretreatments before keeping them for germination.

Pretreatment No. 1.—Enough seeds of all these three species were packed in four muslin cloth pieces forming four packets. Each packet was wrapped in a coarser and stronger piece of cloth. These final packets were buried separately on 19th June, 1954 in soil collected from a pond and kept in four big earthen pots. The pots were flooded with water upto the brim and were maintained in this condition. The first packet was taken out after 8 weeks, the second after 12 weeks, third after 15 weeks and the fourth after 19 weeks. These seeds were kept for germination under moist filter-paper in Petri dishes in the following conditions and the germination result was watched for four months.

Condition "A".—The seeds were kept in a refrigerator at 9° C. for all the time. The result of germination is shown in the following table.

Name of spp.	Seeds treated for 8 weeks %	Seeds treated for 12 weeks %	Seeds treated for 15 weeks %	Seeds treated for 19 weeks %
H. supinum ..	90	70	15	4
P. plebejum ..	90	98	98	98
M. hirta ..	100	98	..	92

Condition " *B* ".—The seeds were kept in the refrigerator at 9° C. for 12 hours during 9 P.M.–9 A.M. and in room condition from 9 A.M. to 9 P.M. every day. The temperature of room during daytime ranged from 31° C. to 20° C. The germination of seeds was as given in the table below.

Name of Spp.		Germination for seeds treated for 12 weeks %	Germination for seeds treated for 15 weeks %	Germination for seeds treated for 19 weeks %
H. supinum	..	80	95	76
P. plebejum	..	90	95	92
M. hirta	..	98	..	100

Condition " *C* ".—The seeds soaked for 19 weeks were kept in the room from 9 A.M. to 9 P.M. and during night were kept outside in the open. The temperature in the open was found to reach 9° C. during the germination experiment because this was done in November. The result is shown in the following table.

Name of Spp.		% Germination
H. supinum	..	80
P. plebejum	..	92
M. hirta	..	96

Condition " *D* ".—The seeds were kept for germination in an oven at a constant temperature of 35° C. The germination percentage was as follows:—

Name of Spp.		% Germination			
		Seeds soaked for 8 weeks	Seeds soaked for 12 weeks	Seeds soaked for 15 weeks	Seeds soaked for 19 weeks
H. supinum	..	70	72	..	40
P. plebejum	..	10	42	..	25
M. hirta	..	0	28	..	100

Condition "E".—Seeds were kept for germination at 5° C. in a refrigerator continuously.

In this condition there was no germination at all, though in case of seeds soaked for 15 weeks they germinated normally when they were brought after 12 days to room condition.

Condition "F".—The seeds were kept at room condition receiving diffuse light during day and no light during night. The temperature of the room ranged between 20° C. and 31° C. during the whole period under observation.

All the seeds failed to germinate under this condition.

Condition "G".—The seeds which were soaked for 19 weeks, were divided in four lots. In course of germination experiment one lot was kept at 9° C. for 4 hours, second lot for 8 hours, third lot for 12 hours and fourth lot for 30 hours and afterwards were removed to room conditions, *i.e.*, to a temperature between 20° C. and 31° C. The result of germination is given in the following table:—

Name of Spp.	At 9° C. for			
	4 hours only *	8 hours only	12 hours only	30 hours only
H. supinum	No germination	No germination	No germination	52% germination
P. plebejum	24% ,,	24% ,,	72% ,,	72% ,,
M. hirta ..	0% ,,	0% ,,	16% ,,	32% ,,

* This set was removed to condition "C" after 10th day of observation for a number of days. In a further time of 8 days a normal germination of all the spp. took place.

The seeds, soaked for 12 weeks, 15 weeks and 19 weeks, were allowed to dry temporarily at the room condition for a few days. Such air-dry seeds were kept for germination at 9° C. during nights and at room conditions during daytime. The result of germination is given below:—

Name of Spp.	Seeds soaked for 12 weeks %	Seeds soaked for 15 weeks %	Seeds soaked for 19 weeks %	Seeds unsoaked %
H. supinum ..	12	56	72	No germination
P. plebejum ..	78	96	84	85 ,,
M. hirta ..	20	..	96	No germination

Thus it appears that *Polygonum* does not show any effect on % germination after being dried temporarily., *Heliotropium* and *Mollugo* show poor germination if dried after soaking for short time but no marked adverse effect of drying is noticeable in case if they are soaked for a longer time.

The rate of germination of soaked seeds:

The rate of germination is shown in the following table under the optimum condition:—

Table to show rate of germination in case of Mollugo hirta

Pretreatment of seeds	Total germination %	No. of days after which germination began	No. of days needed for the full germination
Seeds soaked for 8 weeks	100	12 days	12 days
,, ,, ,, but dried ..	70	11 ,,	34 ,,
Seeds soaked for 12 weeks	98	5 ,,	22 ,,
,, ,, ,, but dried tempy.	20	14 ,,	45 ,,
,, ,, 19 weeks	100	4 ,,	4 ,,
,, ,, ,, but dried tempy.	96	3 ,,	4 ,,

By seeing the graphs it becomes clear that soaking for longer periods hastens the rate of germination in all the cases. The soaked seeds when dried temporarily start germination after long time, especially in case of soaking for shorter period. However, *Polygonum* is not much affected by drying.

2nd Pretreatment.—The seeds of all the spp., which were stored in bottles in room conditions for nearly four months, were treated with conc. sulphuric acid for 1 minute in case of one lot and for 2 minutes in the case of the other lot. After acid treatment the seeds were washed many times with distilled water and were kept for germination under moist filter-paper in Petri dishes, under condition "A", "B", "D", "E" and "F".

Only *Polygonum plebejum* showed 80% germination under "A" and "B", *i.e.*, when exposed to 9° C. and others failed to germinate under any condition.

3rd Pretreatment.—The seeds of all the 3 species which were stored in dry bottles for 4 months were exposed to $-10°$ C. for 7 days and thereafter were kept for germination under the conditions "A", "B", "D", and "E" and "F" as usual.

In this case also only *Polygonum* showed 85% germination under conditions "A" and "B".

4th Pretreatment.—The seeds which were stored in dry bottles for 4 months in room conditions were exposed to a constant temperature of 56° C. for 7 days and thereafter were kept for germination under various conditions as before.

In this case also only *Polygonum* germinated to a total of 85% under conditions "A" and "B" and the other two species failed to germinate at all.

Seeds stored in dry bottles for 4 months and 16 months were kept for germination under various conditions as in previous cases. Only *Polygonum* showed a germination of 85% and 100% respectively in the two cases while the other two did not germinate at all.

Discussion

Theoretically, soaking of seeds accelerates the metabolic processes preparatory to germination. Some grass seeds, especially *Dactylis glomerata*, show better germination of seeds after soaking (Chippindale, 1934). At the same time many seeds have been known which showed definite adverse effect of soaking (Kidd and West, 1918). Tilford and Hibbard (1924) showed an injurious effect of soaking in water in case of *Phaseolus vulgaris*. In the present case the seeds of three species were kept embedded in mud with lot of population of micro-organisms. Such soaking enabled the seeds of *Heliotropium supinum* and *Mollugo hirta* to germinate. The untreated seeds failed to germinate under any condition. Even seeds stored for a year and half showed no germination. Not only that the treatment for a longer period hastened the rate of germination and very likely there may be a minimum period upto which the seeds must be soaked to enable it to germinate.

Polygonum plebejum on the other hand is almost indifferent to this treatment, though soaking definitely accelerates the rate of germination. The seeds of this species germinated even when untreated.

What is the effect of soaking in this manner? It seems probable that soaking in presence of micro-organisms makes the seed-coat permeable and

it may be that the seed-coats in these species are impermeable to water. But even then the question remains whether impermeability alone is responsible for failure in germination. To some extent the answer is got by noting the germination after treating the seeds with conc. sulphuric acid, which is reported to make the seed-coat permeable. Even after this treatment seeds of *Heliotropium supinum* and *Mollugo hirta* did not germinate. Moreover the seeds thus soaked, after being allowed to dry once again, did not germinate readily, and even when they did, germination percentage was much lower. In this case another remarkable point is that the seeds which were soaked for shorter time showed delayed germination.

The effect of low temperature on germination has been known in case of a number of species. *Camassia leichtlinii* Wats. germinate better at 5° C. and *Lewisia rediviva* Pursh. requires a temperature lower than 10° C. for germination (Schroeder and Borton, 1939). A fairly low temperature is necessary for successful germination of celery seeds (Hopkins, 1928). Barton found that seeds of annual *Delphinium* requires low temperature for germination. The effect of low temperature is very marked in case of the three seeds under the present study. All the three species germinate when kept at a temperature of 9° C. The upper limit of the temperature required for germination could not be found but it is lower than 20° C. because under present observation the night temperature in the room touched 20° C. but seeds kept in room temperature failed to germinate. When the seeds were kept in the open during night when the temperature recorded was lower than 9° C., they germinated normally. Even a temperature as low as 5° C. has its positive effect to bring about the germination when such exposed seeds are brought to room temperature because perhaps this temperature proves too low to effect growth.

It seems that for these seeds an exposure to low temperature for certain hours is necessary for germination and *Polygonum* needs it for lesser time and the other two for a longer time. If soaked seeds are exposed to 30 hours *Polygonum* show 72% germination, *Heliotropium* 52% and *Mollugo* 32% and in case of *Heliotropium* seeds exposed to low temperature for less than 12 hours do not germinate at all.

Warington (1936) has shown the effect of alternations in temperature in case of some weed seeds. The effect of alternations in temperature between 9° C. to 31° C. in case of the seeds under the present study is not at all marked as regards the total percentage germination but certainly under such conditions rate of germination is faster.

It is interesting to find that the soaked seeds of *Heliotropium supinum* are able to germinate if kept constantly at such a high temperature as 35° C. It appears that seeds soaked for shorter time show better germination at such a high temperature, while seeds of *Mollugo* if soaked for a long time are able to germinate well at this temperature.

CONCLUSION

The seeds of the 3 species under study show some specific requirements for germination. The seeds of *Heliotropium supinum* and *Mollugo hirta* will not germinate if not soaked in mud for some time. However, for *Polygonum plebejum*, this preconditioning is not at all necessary. All the three species need a low temperature for some minimum period to enable germination. *Heliotropium supinum* can germinate well even at such a high temperature as 35° C. Soaking makes the testa permeable and probably activates the metabolic processes preparatory to germination. Such soaked seeds if dried under room condition for a few days show poor percentage germination and take longer time to germinate.

SUMMARY

1. The effects of some pretreatments on the germination of seeds of *H. supinum*, *P. plebejum* and *Mollugo hirta* have been found out.

2. The temperature needed for the germination of these three seeds has been found out.

3. The effect of temporary drying of soaked seeds at room temperature has been observed.

4. The effect of some pretreatments on the germination has been determined in case of all these three species.

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